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Nuclear Import and Export Pathways

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Abstract Macromolecules enter or leave the nucleus by using nuclear localization signals (NLS), or nuclear export signals (NES), respectively. Different types of NLS and NES are recognized directly or indirectly via adapters, by transport receptors. All transport receptors identified thus far are members of the same family and share an ability to shuttle between the nucleus and the cytoplasm, and to interact with the small GTPase Ran and with nucleoporins at the nuclear pore complex (NPC). The GTPase Ran regulates the interaction of transport receptors with either cargoes, or adapters, or nucleoporins and is crucial in providing directionality to nuclear import and export. Surprisingly, GTP hydrolysis by Ran is not required for translocation of some receptor/cargo complexes through the NPC. One of the challenges for the future will be to establish the mechanisms of translocation through the NPC of different transport receptors together with their cargoes. J. Cell. Biochem. Suppls. 32/33:76–83, 1999.

Key words: karyopherins; nuclear pore complex; nucleoporins; Ran GTPase

The spatial segregation of DNA replication and RNA biogenesis in the nucleus, and protein synthesis in the cytoplasm of eukaryotic cells, requires selective and regulated transport of proteins and RNA between the two compartments. Cargoes enter and exit the nucleus through the nuclear pore complexes (NPC), structures that penetrate the nuclear envelope. The diameter of the NPC channel through which active transport occurs can open to a maximum of 25 nm, whereas the diffusion channel through which transport of ions and small molecules occurs is 9 nm. Macromolecular cargoes carry a nuclear localization signal (NLS), or a nuclear export signal (NES) to be able either to enter or exit the nucleus. The first identified NLS, referred to as classic NLS, fall into two categories: a simple sequence of 3–5 basic amino acid residues (monopartite), and a bipartite signal consisting of a basic dipeptide upstream from a simple basic sequence. Other types of NLS have been identified in hnRNP proteins, ribosomal proteins, and U snRNPs. Leucine-rich NES have been identified in a variety of cellular and viral proteins. Transport signals are recognized by import or export receptors of the karyopherin β (Kap β) (importin/exportin) family, which shuttle between the nucleus and cytoplasm [reviewed by Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998; Pemberton et al., 1998; Moroianu, 1999].

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NUCLEAR IMPORT AND EXPORT RECEPTORS

The basic paradigm for nuclear import is that the NLS cargo is bound either directly, or indirectly, via an adapter, by an import receptor in the cytoplasm, translocated through the NPC, and released in the nucleus. The receptor (and the adapter) is then exported/recycled back to the cytoplasm in a form competent for another round of import.

The first identified nuclear import receptor was Kap β1 [importin β [Imp β], p97, PTAC β; Table I), which functions together with an adapter, Kap α [importin α [Imp α], NLS receptor), in nuclear import of proteins that contain classic monopartite or bipartite NLS (Fig. 1). Kap α binds to the NLS of the cargo, whereas Kap β1 mediates docking at the NPC to nucleoporins that contain peptide repeats. There are several human Kap α, and homologues have been characterized in other eukaryotes. It is likely that different Kap α, have both distinct and overlapping specificity for basic NLS. All Kap α have a common structure: an N-terminal domain rich in basic residues that binds Kap β1 and a central domain composed of 8–10 armadillo-like (ARM) repeats [reviewed by Izaurralde and Adam, 1998; Mattaj and Englmeier,

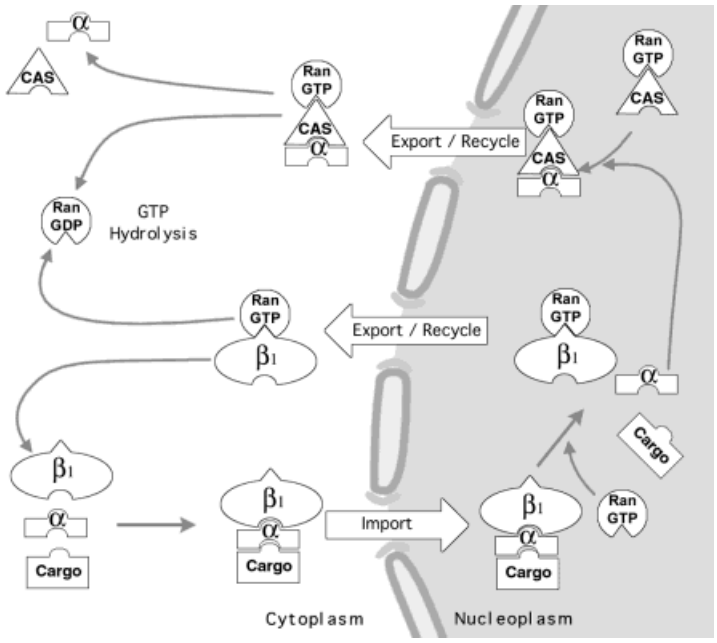

Fig. 1. Model for nuclear import of classic NLS-containing proteins. The NLS-protein cargo binds in the cytoplasm to the Kap αβ1 heterodimer, which is stable because of low RanGTP levels. The resulting trimeric complex docks through Kap β1 to nucleoporins at the cytoplasmic fibers of the NPC and is translocated through the NPC. RanGTP then dissociates the Kap αβ1 complex and thus releases the α subunit and its NLS-protein cargo into the nucleus. After the completion of the import reaction, Kap α and Kap β1/RanGTP are exported through separate pathways into the cytoplasm. Nuclear export of Kap α is mediated by CAS, a member of the Kap β family.

1998; Moroianu, 1998]. Crystallographic analysis of the ARM repeat domain of yeast Kap α bound to an NLS peptide showed that the binding site for monopartite NLS is within the ARM repeats; this finding suggested a model for recognition of bipartite NLS [Conti et al., 1998].

Kap β1 can function with another α-like adapter, called snurportin 1, in nuclear import of U snRNPs. Snurportin 1 has a similar N-terminal binding domain for Kap β1 to that of Kap α, and a C-terminal m3G-cap-binding region with no structural similarity to the ARM repeats of Kap α [Huber et al., 1998]. Kap β1 also functions without adapters in nuclear import of the ribosomal proteins, L23a, S7, and L5 [Jakel and Görlich, 1998], cyclin B1 [Moore et al., 1999], and viral proteins, such as Tat and Rev of human immunodeficiency virus type 1 (HIV-1) [Truant and Cullen, 1999].

During the past years, an entire family of Kap β (importins/exportins) has been identified both in yeast and higher eukaryotes. The Kap β have the highest level of conservation at their N-terminus, a region involved in binding the GTPase Ran [Görlich et al., 1997]. These Kap β bind their cargoes directly with no need for adapter proteins. It has been proposed that there are 14 putative Kap β in yeast [Görlich et al., 1997; see also Pemberton et al., 1998]; several of their vertebrate homologues have been characterized (Tables I, II). Nine yeast Kap β and 6 vertebrate Kap β have been shown to function in nuclear import of specific cargoes (Table I), and four yeast Kap β and 3 vertebrate Kap β have been found to function in nuclear export (Table II).

Yeast Kap104p mediates nuclear import of the mRNA binding proteins Nab2p and Nab4p [Aitchison et al., 1996]. Its vertebrate homologue, Kap β2/transportin 1 (TRN1), mediates nuclear import of hnRNP A1 [Pollard et al., 1996; Bonifaci et al., 1997; Fridell et al., 1997] and hnRNP F [Siomi et al., 1997]. The hnRNP A1 import signal, the M9 domain, is different from the classic basic NLS, rich in glycine and aromatic amino acids [Siomi and Dreyfuss, 1995]. Interestingly, Kap β2/TRN1 is able to interact not only with the M9 signal, but with other types of import signals present in other hnRNP proteins [Siomi et al., 1997] and ribosomal proteins [Jakel and Görlich, 1998] as well.

The third major nuclear import pathway, identified first in yeast, refers to nuclear import of a set of ribosomal proteins mediated by two members of the Kap β family, namely Kap123p and Kap121p [Rout et al., 1997; Schlenstedt et al., 1997]. KAP123 is not an essential gene; this can be explained by the fact that Kap121p can replace the function of Kap123p as a Kap β for nuclear import of yeast ribosomal proteins [Rout et al., 1997]. KAP121 is an essential gene, suggesting that Kap121p might mediate additional nucleocytoplasmic transport pathways. Indeed, Kap121p was also found to mediate nuclear import of the yeast transcription factor

TABLE I. Nuclear Import Pathways Mediated by β-Karyopherins/Importins

β Kaps (+ synonyms) (vertebrate)	β Kaps (+ synonyms) (yeast)	Adapters	Import cargoes
Kap β1 (Imp β, p97, PTAC β)	Kap95p	Kap α, Kap60p (Imp α, PTAC α)	Proteins that have classic basic monopartite or bipartite NLS
		snurportin 1	U snRNPs
		None	Ribosomal proteins; cyclin B; viral proteins
Kap β1 + Imp7		None	Histone $H1^0$
Kap β2/TRN1	Kap104p	None	mRNA-binding proteins;
		None	Mammalian ribosomal proteins
TRN-SR		None	Mammalian SR-proteins
Kap β3/Imp5	Kap121p/Pse1p	None	Ribosomal proteins; yeast Pho4
Kap β4	Kap123p/Yrb4p	None	Ribosomal proteins
(Imp7 or Imp8 ?)[b]	Kap108p/Sxm1p[a]	None	Yeast Lhp1p; ribosomal proteins?
	Kap111p/Mtr10p	None	Yeast mRNA-binding protein, Np13p
(Imp7 or Imp8 ?)[b]	Kap119p/Nmd5p[a]	None	Yeast TFIIS;
		?	Yeast HOG1 MAP kinase
	Kap114p	None	Yeast TATA-binding protein
	Kap122p/Pdr6p	None	Yeast TFIIA
Imp7		None	Mammalian ribosomal proteins

[a]Nmd5p and Sxm1p are both approximately 50% similar to human Imp8.
[b]Imp7 and Imp8 are approximately 63% identical.

TABLE II. Nuclear Export Pathways Mediated by β Karyopherins/Exportins

Export receptors (vertebrate)	Export receptors (yeast)	Export cargoes
CRM1/ exportin 1	Crm1p/Kap124p	Proteins that have leucine-rich NES snurportin 1
CAS	Cse1p/Kap109p	Kap α, Kap60
	Msn5p	Yeast transcription factor Pho4
Exportin t	Los1p	tRNA

Pho4 [Kaffman et al., 1998a]. The lethal phenotype of a KAP121 null mutation can be rescued by the overexpression of SXM1/KAP108 [Seedorf and Silver, 1997]. Kap108p/Sxm1p was found to interact with three ribosomal proteins, suggesting that it might be involved as a backup Kap in importing these proteins [Rosenblum et al., 1997]. Conditional loss of Kap121p in a strain lacking Kap123p results in a block of mRNA export from the nucleus, suggesting an involvement (perhaps indirect) of these Kap in mRNA export [Seedorf and Silver, 1997]. The mammalian homologue of yeast Kap121p, Kap β3 (also called Imp5; see Table I), binds to ribosomal proteins [Yaseen and Blobel, 1997] and was shown to function in nuclear import of the ribosomal proteins L23a, S7, and L5 [Jakel and Görlich, 1998]. Interestingly, each of these ribosomal proteins can bind directly, and be imported by, at least four Kap β: Kap β1, Kap β2, Kap β3, and Imp7. The NLS of the ribosomal protein L23a recognized by these four Kap β is very basic and is much more complex (42 amino acids) than a classic NLS. The binding sites in Kap β2/TRN1 for this NLS and for the M9 domain are distinct [Jakel and Görlich, 1998].

Yeast Kap111p/Mtr10p binds directly to the shuttling mRNA-binding protein, Npl3p and transports it into the nucleus [Pemberton et al., 1997]. Yeast Kap108p/Sxm1p binds directly and transports into the nucleus Lhp1p, a protein that functions in tRNA maturation [Rosenblum et al., 1997]. In addition, through its association with three ribosomal proteins (Rpl16p, Rpl25p, Rpl34p), Kap108p/Sxm1p may have a role in coordinating ribosome biogenesis with tRNA processing [Rosenblum et al., 1997]. It is unclear which is the vertebrate homologue of yeast Kap108p/Sxm1p. Both Imp7 and Imp8 have similar homology with Kap108p/Sxm1p and with Kap119p/Nmd5p (Table I). Imp7 has been shown to be one of four Kap β that mediate nuclear import of several ribosomal proteins [Jakel and Görlich, 1998]. Interestingly, Kap β1, together with Imp7, mediates nuclear import of the linker histone H1. Individually, the two import receptors bind H1 weakly, but binding is strong for the Kap β1/Imp7 heterodimer

[Jakel et al., 1999]. This is the first example in which 2 Kap β cooperate in binding and nuclear import of a cargo.

Yeast Kap119p/Nmd5p was shown to mediate nuclear import of the transcription elongation factor TFIIS [Albertini et al., 1998]. Cytoplasmic Kap119p exists in an approximately stoichiometric complex with TFIIS. In a strain in which KAP119 has been deleted, nuclear TFIIS was mislocalized to the cytoplasm, whereas the localization of other cargoes transported by Kap60p/Kap95p, or Kap108p, or Kap111p, or Kap104p was not affected. Interestingly, Kap119p/Nmd5p is also required for the nuclear import of the MAP kinase HOG1 that occurs in response to high osmolarity of the growth medium [Ferrigno et al., 1998].

Yeast Kap114p binds and transports into the nucleus the TATA-binding protein (TBP), a factor involved in transcription of genes by all three RNA polymerases [Pemberton et al., 1999]. Deletion of the KAP114 gene leads to specific mislocalization of TBP to the cytoplasm. Kap114p is not an essential protein, and two additional Kap β, Kap123p and Kap121p, might participate in TBP nuclear import [Pemberton et al., 1999]. Like yeast and mammalian ribosomal proteins [Rout et al., 1997; Jakel and Görlich, 1998], and yeast TFIIA [Titov and Blobel, 1999], TBP is an essential protein that appears to enter the nucleus through multiple Kap-mediated pathways.

Transportin-SR (TRN-SR), a mammalian Kap β, mediates nuclear import of arginine/serine (RS)-rich proteins, which are essential pre-mRNA splicing factors [Kataoka et al., 1999]. TRN-SR binds specifically and directly to the RS domains of ASF/SF2 and SC35 as well as to other SR proteins and mediates their nuclear import. TRN-SR is 25% identical and 46% similar to ALO22304 of *Schizosaccharomyces pombe* (although the clone does not appear to contain the full length protein sequence), and 21% identical and 42% similar to Kap111p/Mtr10p.

Four members of Kap β family have been identified to function in nuclear export of specific cargoes (Table II). CAS was identified as a Kap β, which binds Kap α in a RanGTP-dependent manner and is involved in its nuclear export/recycling [Kutay et al., 1997](Fig. 1). Cse1p, the yeast homologue of CAS, mediates nuclear export of Kap60 [Hood and Silver, 1998].

Crm1p or exportin 1 (Xpo1p) has been identified in both yeast and higher eukaryotes as a member of the Kap β family that binds leucine-rich NES in a RanGTP-dependent manner and mediates nuclear export of NES-containing cargoes [Fornerod et al., 1997; Kudo et al., 1997; Ossareh-Nazari et al., 1997; Stade et al., 1997].

The first identified cellular RNA-export receptor, called exportin-t in higher eukaryotes or Los1p in yeast, mediates nuclear export of tRNA. Exportin-t binds tRNA directly in a RanGTP-dependent manner and it is rate limiting for tRNA nuclear export in microinjection experiments [Kutay et al., 1998; Arts et al., 1998].

Msn5p, a yeast Kap β, is required in vivo for nuclear export of the transcription factor Pho4 [Kaffman et al., 1998b]. Interestingly, Msn5p binds only to phosphorylated Pho4 in the presence of RanGTP and translocates it through the NPC to the cytoplasm [Kaffman et al., 1998b]. This constitutes an efficient way of regulating nucleocytoplasmic transport of a transcription factor via phosphorylation. Dephosphorylated Pho4 is imported into the nucleus through its interaction with Kap121p/Pse1p [Kaffman et al., 1998a]. In the nucleus, phosphorylation of Pho4 by a nuclear complex of a cyclin with a cyclin-dependent kinase, Pho80-Pho85, triggers its nuclear export via Msn5p [Kaffman et al., 1998b]. The ability of Msn5p to recognize phosphorylated Pho4 could be explained either by a conformational change triggered by phosphorylation that would reveal the Msn5p-binding site, or alternatively, Msn5p may recognize a phosphopeptide within Pho4. In this case Msn5p may function as a general export receptor for phosphorylated proteins [Kaffman et al., 1998b].

ROLE OF RAN IN NUCLEAR IMPORT AND EXPORT

All Kap β (importins/exportins) have in common the ability to interact with Ran in its GTP-bound state. The RanGTPase cycle acts as a molecular switch for nucleocytoplasmic transport. An important feature of the RanGTPase system is the cellular distribution of its regulators. The main GTPase-activating protein, RanGAP, and a Ran-binding protein, RanBP1, which together can achieve maximal GTPase activity, are mainly cytoplasmic. By contrast, the nucleotide exchange factor of Ran, RCC1, is mainly nuclear and is bound to chromatin. As a result of this distribution there is a concentration gradient of RanGTP across the NPC: low in the cytoplasm and high in the nucleus [reviewed by

Izaurralde and Adam, 1998; Mattaj and Englmeier, 1998; Moroianu, 1999].

RanGTP interacts with Kap β/importins (Kap β1/Kap 95p, Kap β2/Kap104p, Kap β3/Kap121p, Imp7, Kap111p, Kap114p, Kap119p) and this causes dissociation of the import complexes inside the nucleus with formation of RanGTP/Kap β complexes [Rexach and Blobel, 1995; Moroianu et al., 1996; Görlich et al., 1997; Jakel and Görlich, 1998; Albertini et al., 1998; Ferrigno et al., 1998; Chook and Blobel, 1999; Pemberton et al., 1999]. The RanGTP gradient across the NPC controls the formation of Kap import complexes: assembly occurs in the cytoplasm (low RanGTP) and disassembly in the nucleus (high RanGTP) (Fig. 1). This regulation of cargo (or adapter) binding to import receptor and release by the nucleotide state of Ran is a critical function of Ran that provides (at least partially) directionality of nuclear import. The structural asymmetry of the NPC might also contribute to the directionality of transport. An increasing affinity binding gradient of import receptors for nucleoporins along the NPC with the highest on the nucleoplasmic side, and an opposite gradient for export receptor/RanGTP complexes with the highest on the cytoplasmic side of the NPC would favor directionality in import and export, respectively.

An important question is how binding of RanGTP to Kap β promotes the release of the cargo. Recently, the structures of two import receptors, Kap β1 and Kap β2, in complex either with Ran loaded with the nonhydrolyzable GTP analogue GppNHp, or with the cargo have been determined [Chook and Blobel, 1999; Cingolani et al., 1999; Vetter et al., 1999]. The structures of Kap β1 and Kap β2 show a similar series of helical HEAT repeats (19 in Kap β1 and 18 in Kap β2) and an acidic intra-HEAT loop (L8 in Kap β1 and L7 in Kap β2). In the structure of a complex between Ran and a 462 residue fragment of Kap β1, the N-terminal HEAT repeats surround Ran and the acidic intra-HEAT loop L8 makes several contacts with Ran [Vetter et al., 1999]. The N-terminal Kap β1 binding domain of Kap α interacts with residues on the β helices of the C-terminal HEAT repeats 7–19, and with the acidic intra-HEAT loop L8 of Kap β1 [Cingolani et al., 1999]. Deletion studies have previously suggested that the acidic loop in Kap β1 (residues 329–342) is part of both the Ran-binding domain and Kap α-binding domain, as removal of these residues decreased the affinity of Kap β1 for both Ran and Kap α [Moroianu et al., 1996]. As the acidic loop of Kap β1 interacts both with Ran and Kap α, binding of Ran and Kap α to the loop will be mutually exclusive. The structure of Kap β2/Ran complex shows that about 62% of the Ran interaction surface is contributed by the acidic loop L7, which is very long (62 amino acids), and the rest by the N-terminal HEAT repeats [Chook and Blobel, 1999]. The M9-binding site was previously mapped to the C-terminal HEAT repeats of Kap β2 with no requirement of the acidic loop [Pollard et al., 1996; Fridell et al., 1997]. Interestingly, the structure of Kap β2/Ran suggests a mechanism of cargo dissociation in which Ran binding to Kap β2 is accompanied by an allosteric conformational change in the long acidic loop L7 that puts the loop into the cargo site and therefore displaces the cargo [Chook and Blobel, 1999]. For the Kap αβ1 heterodimer it remains to be established if an allosteric conformational change induced by Ran-binding contributes to the mechanism of Kap α dissociation.

Whereas in nuclear import RanGTP triggers the release of the cargo into the nucleus, in export the presence of RanGTP in the nucleus permits the formation of the cargo/export receptor/RanGTP trimeric complexes. All Kap β involved in nuclear export (CAS, exportin 1/Crm1p, exportin t, Msn5p) bind their specific cargoes preferentially in the presence of RanGTP [Kutay et al., 1997; Fornerod et al., 1997; Stade et al., 1997; Kaffman et al., 1998b]. The export complexes are translocated through the NPC to the cytoplasm, where GTP hydrolysis through the combined action of RanGAP and RanBP1 results in dissociation of Ran from the complex. Thus, the export receptor is able to release its cargo [Kutay et al., 1997].

The Kap β (importins/exportins) exit the nucleus as complexes with RanGTP, and therefore constantly deplete Ran from the nucleus. Ran is actively reimported into the nucleus via the transport factor p10/NTF-2, which binds specifically to RanGDP [Ribbeck et al., 1998; Smith et al., 1998]. The release of Ran from p10/NTF-2 probably involves nucleotide exchange to generate RanGTP, for which p10/NTF-2 has no detectable affinity.

Recent studies regarding the role of GTP hydrolysis by Ran in nucleocytoplasmic transport have changed our way of thinking about the use of energy in translocation of receptor/cargo complexes through the NPC. Data from these studies, which used recombinant trans-

port factors and nonhydrolyzable GTP analogues, showed that GTP (or NTP) hydrolysis by Ran is not required for karyopherin α2β1-mediated translocation of a classic NLS cargo through the NPC into the nucleus [Schwoebel et al., 1998]. Careful analysis of single nuclear import events mediated by either Kap β2/TRN1, or snurportin1-Kap β1, or single nuclear export events of an NES cargo mediated by Crm1, indicated that they do not require GTP (or NTP) hydrolysis. Apparently, hydrolysis of GTP by Ran is required only for recycling the Kap β for multiple rounds of transport [Ribbeck et al., 1999; Englmeier et al., 1999].

INTERACTIONS BETWEEN NUCLEAR TRANSPORT RECEPTORS AND NUCLEOPORINS

The NPC consists of three rings (cytoplasmic, central, and nucleoplasmic), eight filaments that extend from the cytoplasmic ring, a central transporter, and a "nuclear basket" formed by eight nucleoplasmic filaments that extend from the nuclear ring and are joined to a small fourth ring. The cytoplasmic filaments of the vertebrate NPC contain the nucleoporins Nup358 and Nup214, both of which have FXFG peptide repeats. A complex of nucleoporins, p62/58/54/45 has been localized in the region of the central transporter, through which nucleocytoplasmic transport occurs. The nuclear basket contains the nucleoporins Nup98 and Nup153, which have peptide repeats [reviewed by Doye and Hurt, 1997].

Many interactions between Kap β and nucleoporins have been reported for both the vertebrate and yeast transport systems. Overlay blot assays and co-immunoisolation experiments have suggested that vertebrate Kap β (Kap β1, Kap β2, Kap β3, CRM1) can bind overlapping sets of nucleoporins [Moroianu et al., 1995; Bonifaci et al., 1997; Yaseen and Blobel, 1997; Fornerod et al., 1997; Shah et al., 1998; Nakielny et al., 1999]. Biochemical and functional analysis of the interactions between Kap β1, Kap β2, and Nup153 showed that the Kap β1 and Kap β2-mediated nuclear import pathways converge on Nup153. Interestingly, Nup153 contains separate binding sites for Kap β1 and Kap β2—an FG peptide repeat fragment that binds specifically Kap β1, and an N-terminal fragment that binds specifically Kap β2 [Shah and Forbes, 1998; Nakielny et al., 1999]. The Nup153/Kap β2-binding site is an M9 shuttling domain that confers to Nup153 the ability to move within the NPC [Nakielny et al., 1999].

Nearly 30 yeast nucleoporins have been characterized, many of which contain FG peptide repeat domains [reviewed by Doye and Hurt, 1997]. Members of the yeast Kap β family (Kap95p, Kap104p, Kap123p, Kap121p, Kap111p, Kap119p) have been shown to interact with FG peptide repeat-containing nucleoporins. Individual yeast Kap β can bind separate but overlapping subsets of nucleoporins with different affinities [Rexach and Blobel, 1995; Aitchison et al., 1996; Pemberton et al., 1997; Rosenblum et al., 1997; Rout et al., 1997; Albertini et al., 1998; Marelli et al., 1998]. It remains to be established for each individual Kap β and nucleoporin, the binding sites and the functional significance of the interaction for the respective nuclear import or export pathway.

An important aspect of nucleocytoplasmic transport is that Ran is involved, at least in some cases, in the control of Kap β- nucleoporin interactions. Binding of RanGTP to several import receptors (Kap β1/Kap95p, Kap β2, Kap121p) dissociates the receptor from the nucleoporin [Rexach and Blobel, 1995; Shah et al., 1998; Shah and Forbes, 1998; Marelli et al., 1998; Nakielny et al., 1999]. It is not clearly established if during nuclear import RanGTP dissociates the import receptors from nucleoporins at multiple locations within the NPC or only on the nucleoplasmic side of the NPC, and if there are differences between different Kap β. In the classic import pathway, RanGTP-mediated disassembly of the NLS cargo/Kap αβ1/Nup153 complexes releases the free NLS cargo, Kap α and Kap β1-RanGTP from Nup153 [Shah et al., 1998]. Interestingly, in NES mediated export the opposite seems to be true: RanGTP is required for Nup153-CRM1 interactions [Nakielny et al., 1999]. Therefore, Nup153 on the nucleoplasmic side of the NPC could play essential roles in initiating nuclear export and in termination of nuclear import. The zinc-finger domains of Nup153 have affinity for RanGDP, but not with RanGTP [Nakielny et al., 1999]. This finding suggests that Nup153 could also serve as a site at which the imported RanGDP (in complex with p10/NTF-2) would first undergo nucleotide exchange under the action of nuclear RCC1 to generate RanGTP, and as a consequence release p10/NTF-2 at the nucleoplasmic side of the NPC.

RanGTP stimulates binding of Kap β1 to Nup358 at the cytoplasmic side of the NPC,

probably by mediating the binding of the complex to the Ran binding domains of Nup358 [Delphin et al., 1997]. The interaction between Nup358 and different Kap β/RanGTP complexes could constitute the termination step in nuclear export. In this scenario, Nup358 could serve as a common site where the exported Kap β/RanGTP complexes would dock via RanGTP and be hydrolyzed.

CONCLUSIONS AND PROSPECTS

Despite great progress made in the nucleocytoplasmic field during the past few years, major questions remain. Many import and export receptors and the cargoes they transport have been discovered in higher eukaryotes and yeast. Several metazoan Kap β are still awaiting characterization and identification of their cargoes. Also, major classes of cargoes (i.e., mRNPs, ribosomal subunits) are awaiting the discovery of their export receptors. With the continuing identification and characterization of new transport receptors, adapters and import and export signals we will have a more complete picture of the complexity of nucleocytoplasmic traffic. Surprisingly, GTP hydrolysis by Ran is not required for single-round nuclear import or export events through the NPC, and it may be required only for recycling of import receptors or dissociation of export complexes. It remains to be established if the energy derived from transport receptor recycling is used to drive import or export pathways against a concentration gradient. The structures of two import receptors, Kap β1 and Kap β2 in complex with Ran, or cargo (for Kap β1) have been solved, bringing insight into the mechanism of RanGTP-mediated dissociation of import complexes. It remains to be established how binding of RanGTP to export receptors increases, rather than disrupts, cargo binding. The big challenge for the future is to understand the mechanisms of translocation through the NPC of different receptor/cargo complexes during import or export, as well as how these nucleocytoplasmic pathways are regulated. This will require a detailed analysis of the interactions between individual Kap β and nucleoporins (affinity, binding sites, functional significance for import/export, RanGTP regulation), elucidation of the structure of different combinations of Kap β /nucleoporin domains at atomic resolution, and determination of the complete three-dimensional molecular architecture and functional dynamics of the NPC.

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